Melatonin Lowers Levels of SOD and Number of Inflammatory Cells 
**BAL Wistar strain** Mice Wearing Mask PPE, Sub Acute Exposed by Coal Dust Day and Night

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**ABSTRACT**

Respiratory disease caused by exposure to coal dust particles generally follow the pattern of inflammatory oxidative stress. Respiratory disease caused by coal dust can be minimized by wearing dust mask as Personal Protective Equipment (PPE). This study aims to determine the effect of exogenous melatonin administration in masked mice wistar strain with coal dust exposed day and night. The research method by dividing the mice into 5 groups, 1) control group, 2) exposed without melatonin administration , 3) exposed and melatonin 0.5 mg/kg/day, 4) exposed by melatonin 1 mg/kg/day and 5) exposed and administering melatonin 2 mg/kg/day. The study was conducted in two phases, phase I for exposure during the day and phase II for night exposure where the cage was covered by cloth as mask when coal dust exposed for 3 days of each 30 minutes. Melatonin administered for 6 days prior to the dust exposure. The results showed that coal dust exposure lead to significantly increased levels of SOD BAL day 55.662 ± 125 .115 Unit/100 g and night 38.162 ± 161.005 unit/100g. Lung histopathology structural damage caused by exposure to coal dust subacute indicated by an increase in the number of alveolar inflammatory cells during 0842 ± 0545 ± 1.476/field and night 1.466/field. Conclusion there is no significant difference value day or night exposure , which is thought to be caused by the lack of mice adaptation as nocturnal animal against the natural human shifts. Exogenous melatonin dose of 2 mg /kg/day proven effective as an antioxidant against oxidative stress and lung inflammation caused by coal dust exposure. And PPE mask specifically made for mice was able to inhibit the coal dust particles.

**Keywords:** Coal dust, inflammation of lung tissue, SOD, exogenous melatonin, mask

**INTRODUCTION**

Respiratory diseases caused by inhale hazardous substances in the environment are into serious problems. In the United States an estimated 2.4 million workers are exposed to crystalline silica dust or asbestos in the area of mining and non-mining. And 5% suffer from respiratory diseases, of the 100,000 workers. (Speizier, 2005). Concentration of dust in coal mines was 1.17 mg/m3 (Armutcu et al., 2007). Classification of coal mine dust exposure from a low concentration (0.5 mg/m3) to a high concentration (12.3 mg/m3) (Altin et al, 2004b) Air pollution particles PM (Particulate Matter) 10 around the house has been done in the district of Banjar in South Kalimantan (Hamidi, 2000). In this case it was found breathing problems in 55% of infants and toddlers who inhabit the house with dust exposure levels >μ70 g/m3, only 28% infants and toddlers are experiencing respiratory problems at home with μ70< g/m3 dust exposure So the risk of respiratory problems for the concentration of PM 10 in the dust >μ70 g/m3 is 4.75 times

Air quality in the vicinity of the coal mines of PT BHP (Broken Hill propietary) Arutmin Mine Satui, the parameter levels of SO2 and NO2, has exceeded the threshold limit, there is a significant relationship between the percentage increase in pollution to increased coal mining production (Nugroho, 2001) . Diseases caused by chronic exposure to coal dust such as pneumoconiosis include simple coal workers (CWP), progressive massive fibrosis (PMF), chronic bronchitis and emphysema (Armutcu et al., 2007). CWP risk group to not only includes workers who were active in the mines, but also leave nine workers and former workers (Hu et al., 2003; Zhang et al., 2002). Respirable coal dust causes inflammation in the miner lung. Pulmonary inflammation and parenchymal fibrosis changes due to inhaled mineral dust in the lungs (Borm PJA, Schins et al, 1996 ). The process of free radicals formation (ROS / reactive oxygen species) due to exposure to coal dust may occur passively due to mechanical coal particles breakdown or active through biological inflammatory reaction (Dalal et al., 1988).

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Increased levels of SOD (superoxide dismutases) in plasma is also associated with higher levels of silica in the coal even at low exposure concentrations (Kuempel et al., 2003). Miner working in shift pattern day and night shift. Shift work pattern can change circadian rhythms associated with melatonin hormone. Shift work have risks of cardiovascular disease, metabolic disorder risk such as obesity, increased lipid contents, impaired glucose balance and decreased contents of antioxidants and other metabolic syndrome (Knutsson A, 2003). Working in the night shift circadian rhythms of the body that normally diurnal (Kayumov L, et al,2005) will be transformed into nocturnal (Harrington, 1994). Disruption of circadian rhythm could lead non synchronized natural functions that affect body health. (Ghiasvand, 2006). Circadian rhythm directly influence the normal levels natural melatonin in the body. Melatonin is a hormone that naturally found in animals, humans and other organisms (Reiter et al, 2001).

Problems

Coal mining has day and night shift. At night shift miner will experience fatigue because the body has naturally sleep hours, it is necessary increase immune system by administering exogenous melatonin as antioxidants in inhibition of lung damage caused by coal dust exposure, and the use of masks to minimize inhalation of coal dust especially the night shift.

Problem solving is the use PPE such as mask as a way to minimize the incidence of occupational diseases. Conducted administrative control such as the use of Personal Protective Equipment masks to minimize coal dust inhalation while working.

The use of PPE such as masks to minimize inhaling coal dust while working. Melatonin as an antioxidant and anti-inflammatory to cope with oxidative stress caused by exposure to coal dust and circadian rhythm disruption. (Kuempel ED, et al.1995).

Research objective is to determine the effect of the use of PPE and melatonin in maintaining function lung that exposed to coal dust by measuring superoxide dismutase (SOD) and number of pulmonary alveolar inflammatory cells.

METHODS

This study is an experimental research design was Randomized Posttest Control Group Design. Total population of 72 individuals (36 +36 during the night) wistar strain male mice aged 4-6 months, weighing mice 300-500 g, were divided into three groups, negative control, exposure and exposure during the night. Each exposure group will be further divided into four experimental groups, the positive control group, the first dose of melatonin (0.5 mg/kg/hr), dose 2 (1 mg/kg/hr) dose 3 (2mg/kg/hr).

How to Work and Data Collection

Melatonin administration.

Melatonin administration (dissolved in distilled water) using intra gastric feeding tube every day for 6 days according dose of the treatment.

Coal Dust Exposure

Exposure to coal dust carried out for 3 days at 09.00 (day exposure) and 21.00 (night exposure) using a set of exposure (Figure 1.) Each exposure 15 grams coal dust ( ≤ 50 µ) and mice put into set of exposure that covered by gauze as PPE mask for 15 minutes.

Figure 1. Schematic set exposure
Taking BAL and lung tissue after exposure of mice for 3 days, 16-36 hours later prepared sample is taken. BAL fluid inserted into the tube and seal tightly, centrifuge at 3500 rpm for 10 min at 4 °C. Supernatant was used for the sample (SOD and the number of inflammatory cells) Having taken BAL fluid, lung tissue was fixed in 10% formalin, and then made preparations histopathology with HE staining.

Examination of SOD BAL
Measurement of SOD activity based on the inhibition of SOD working through reduction of NBT by xanthine-xanthine oxidase system (Subandi, 1998)

Examination of the number of inflammatory cells
Manually count the number of inflammatory cells (according to the characteristics of leukocytes) with 400X magnification using a light microscope (40x objective lens, ocular lens 10x) using oil emersi. At 20 field of view. (Leigh R. et al., 2002).

RESULTS

1. The results of measurements of SOD BAL

![Graph showing results of SOD BAL measurements](image)

Figure 2. Comparison of average exposure levels of SOD in BAL day or night

Measurement of SOD levels in BAL showed an increase in levels of SOD in BAL alveolar tissue of mice. The object being measured in this study is the extracellular SOD (EC-SOD) contained in the network. According to (Chow CW, et al, 2003), the majority of the EC-SOD expressed in several tissues including vascular tissue, lung, and uterus. EC-SOD is composed of 70% of the total SOD levels in BAL in humans. SOD is an enzyme of the most powerful natural antioxidants. SOD single cell can deliver signals to other cells in order to produce more SOD, and reactivate and mobilize all the forces antioxidant defense system, including the secondary antioxidants (V Carillo, 2004). This condition becomes very important when the body receives exposure to pollutants that could potentially increase the amount of free radicals.

Normally, in the body there is always the free radicals produced by the endogenous cell organs such as mitochondria and lysosomes. Increasing the number of free radicals in the body usually due to environmental factors. The formation of free radicals caused by exposure to coal dust could be the result of phagocytosis of particles deposit, the particles induced chemically or a combination of both processes. Alveolar macrophages and PMN cells that are responsible for the process of phagocytosis (Rego et al., 1991). If the process of phagocytosis was not able to eliminate the deposit of particles, the ability of antioxidants to eliminate free radicals in the lungs exceeded. This condition will trigger lipid peroxidation and cell damage (Weiss & Lo, 1982; Halliwell, 1999).
2. The results of the measurement number of inflammatory cells in BAL.

![Figure 3. Comparison of the average number of inflammatory cells in BAL exposure of day or night.](image)

The results showed that there was a significant increase ($p = 0.008 <$) the mean number of inflammatory cells in BAL mice exposed to coal dust (positive control) was 1.476 ± 0.318 compared to mice not exposed to coal dust during the day (negative control) was 0.842 ± 0.047. Similarly, a significant increase ($p = 0.002 <$) the mean number of inflammatory cells in BAL mice exposed to coal dust (positive control) was 1.466 ± 0.304 compared to mice not exposed to coal dust in the evenings (negative control) was 0.545 ± 0.185. Researchers (Vallyathan et al, 2000), which exposed coal miners found increased number of inflammatory cells than the control group. Besides, the research conducted (Huang X et al, 2005) an increase in the number of inflammatory cells in rats exposed to coal dust for 48 hours.

Administration melatonin in mice exposed to coal dust can be inhibited subacute increase in the number of inflammatory cells in the BAL. So it can be concluded that the role of melatonin as an antioxidant to inhibit an increase in the number of inflammatory cells in BAL mice exposed to coal dust sub acute.
Melatonin is a fat-soluble molecule and water that can penetrate all cells and cell organelles. Melatonin is a free radical binding very strong for being able to dispose of OH radicals, peroxynitrite anion (ONOO⁻), singlet oxygen (1O₂), O₂ radicals and peroxyl radical (LOO·). This process is more focused on preventing the formation of free radicals than radicals neutralization process that has been established (Hardeland & Poeggeler, 2005).

Decrease in inflammatory cells in the BAL was also associated with NF-κB. NF-κB is a transcription factor that plays a role in the regulation of a number of genes involved in immune and inflammatory processes. Cytosolic NF-κB can be activated by a variety of mediators, including oxidants and viral proteins, which results in the formation of reactive oxygen.). Melatonin is proven to reduce NF-κB binding to DNA, possibly by preventing the translocation of NF-κB to the nucleus. In addition, melatonin can also suppress leukocyte adhesion to endothelial cells and suppress leukocyte migration through endothelial cells that melatonin is shown to reduce PMN recruitment of inflammatory cells into inflamed areas (Reiter et al., 2000).

CONCLUSIONS AND SUGGESTION

Conclusion
Subacute exposure to coal dust in the afternoon and evening increases the levels of activity of superoxide dismutase (SOD), number of inflammatory cells in bronchoalveolar lavage (BAL) mice significantly. And use of PPE mask was found to minimize the risk of occupational diseases in the workplace in the form of coal dust.

As well as giving melatonin proven to prevent damage to the structure of the lung histopathology.

Suggestion
a. Other studies using human or animal diurnal.
b. Exposure method can be changed due to the effect of melatonin chronic methods seem optimal suspected chronic conditions.
c. Melatonin dose variation also needs to be reconsidered given the effectiveness of new looks at the dose of 2 mg / kg / day.
d. Considering the potential of exogenous melatonin as antioxidant intake looks promising, these prospects need to be further investigated in the framework nutriproteomic and nutrigenomics approach.
e. Need to test the effectiveness and efficiency of utilization of exogenous melatonin as antioxidant intake through comparison with vitamins C and E, which have been known. Comparisons can be made in a variety of doses

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